

## PHENOTYPIC MODULATION OF HUMAN ARTICULAR CHONDROCYTES BY BISTRATENE A

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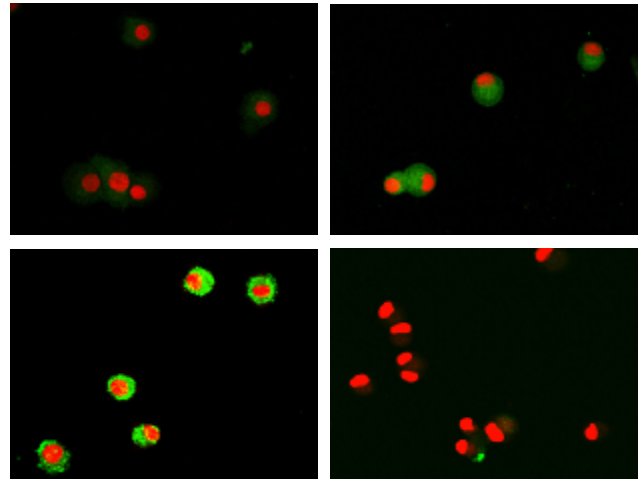
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**INTRODUCTION:** Articular chondrocytes undergo phenotypic alterations following extended periods in monolayer culture, i.e. they become “fibroblast-like” cells, proliferate, and synthesise type I as opposed to type II collagen. This process has been termed chondrocyte dedifferentiation. Such expansion of monolayered chondrocyte cultures is utilized by clinicians for autologous transplantation procedures in the repair of damaged cartilage<sup>1</sup>. However, for such a tissue repair strategy to work, it is necessary for dedifferentiated chondrocytes to “redifferentiate” and again adopt a mature chondrocyte phenotype. Bistratene A is a macrolide polyether that induces morphological changes and differentiation of a number of cell types (including cell rounding of fibroblasts and maturation of melanocytes) through activation of the delta isoform of protein kinase C (PKC $\delta$ ). Here, we report the response of dedifferentiated human articular chondrocytes to treatment with bistratene A.

**METHODS:** Human articular chondrocytes were obtained from macroscopically normal cartilage by collagenase digestion and placed into monolayer culture (for 2-4 passages) to promote their dedifferentiation. Cultures were then treated with 100ng/ml bistratene A or with carrier alone and chondrocyte morphology, cell viability and proliferation assessed over a 5-day time course. Parallel cultures were treated with 100ng/ml bistratene A in combination with the PKC $\delta$ -specific inhibitor, rottlerin. Activation of PKC $\delta$  is associated with translocation of the enzyme to the nuclear membrane<sup>2</sup>. Immunolocalisation for PKC $\delta$  at early time points post-treatment, and for collagen (types I and II) in harvested cells at experimental end-points, were performed. The presence of F-actin stress fibres was determined using FITC-labelled phalloidin.

**RESULTS:** Dedifferentiated human articular chondrocytes became rounded and underwent cell growth arrest after treatment with bistratene A. Bistratene A-treated chondrocytes also became more immunopositive for type II collagen, but less immunopositive for type I collagen (Figure 1). These phenotypic changes were associated with a

prior and extensive disruption of actin microfilaments and translocation of PKC $\delta$  to the nuclear membrane. Concurrent treatments of chondrocytes with rottlerin partially blocked the morphological effects of bistratene A.



*Fig. 1: Human articular chondrocytes become more immunopositive for collagen type II, and less immunopositive for collagen type I, following treatment with bistratene A. (Top panels=collagen II, bottom panels=collagen I: left panels =control cells, right panels=bistratene A-treated cells).*

**DISCUSSION & CONCLUSIONS:** Signalling mechanisms that regulate articular chondrocyte phenotype are likely to be of great importance to tissue engineering strategies for cartilage repair, but are largely unknown. The bistratene A-induced alterations in chondrocyte behaviour reported here may provide a new model system to study an as yet unresolved pathway, potentially involving cytoskeletal and signalling components, that regulates chondrocyte differentiation in vitro.

**REFERENCES:** <sup>1</sup>Richardson J.B., Caterson B., Evans E.H. et al (1999) *J. Bone Joint Surg. Br.* **81**,1064-1068. <sup>2</sup>Griffiths G., Garrone B., Deacon E. et al (1996) *Biochem. Biophys. Res. Commun.* **222**, 802-808.

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